Master of Science in Epidemiology in the field of Epidemiology and Biostatistics

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Research Title:

The pattern of transaminase abnormality among HIV and HBV Co-Infected women on ART in Lilongwe, Malawi

Supervised

By

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DECLARATION

I, Elizabeth Kamwendo Kachingwe declare that this research report is my own work, compiled under the supervision of Professor. Charles. S. Chasela. The report is being submitted to the University of the Witwatersrand in partial fulfilment of a degree of Master of Science in the field of Epidemiology and Biostatistics. There are no prior submissions of this material to other institutions for academic purposes.

Signature

Date:

DEDICATION

To Goodwill, Sean and Tamia

ACKNOWLEDGEMENTS

I would like to thank my family for their support during my studies. Special thanks go to the University of North Carolina (UNC), the Centres Disease Control, Kamuzu Central Hospital and the Breast-Feeding Antiretroviral Nutritional (BAN) study team, for their hard work in collecting the data used in this study.

I would also like to thank all of my lecturers, MSc Epidemiology and Biostatistics program for their dedication and availability to their students. Their teaching prepared me for the process of writing this report and without their continued support it would not have been possible. Special thanks to Prof Levin for his support from the beginning. I would also like to thank my Supervisor, Prof Charles Chasela for his guidance during the writing of this report. His help, expertise and encouragement was invaluable.

Lastly all the praise goes to God almighty, for I can do all things through Christ who strengthens me.

ABSTRACT

Background

Hepatitis B and ART have been established to cause liver damage. We compared the changes in the levels of Alanine amino Transferase (ALT) in HBV/HIV co-infected and HIV infected women on ART to determine liver disease among women on ART in Lilongwe Malawi using Data from the BAN study.

Methods

We conducted a secondary data analysis from The BAN study to investigate the changes in the levels of ALT among HIV/HBV co-infected and HIV mono-infected women who were randomised into the maternal ART arm. In brief The BAN study assessed the benefit of nutritional supplementation given to women during breastfeeding, the benefit and safety of antiretroviral medications given either to infants or to their mothers to prevent HIV transmission during breastfeeding and the feasibility of exclusive breastfeeding followed by early, rapid breastfeeding cessation. ALT was monitored up to 48 weeks with an average of 12 follow-ups per individual. Continuous variables i.e. Age, ALT and CD4 count were compared between HIV/HBV co-infected women and HIV mono-infected women using the Wilcoxon rank sum test. Multiple regression analyses were performed using longitudinal data Generalised Linear mixed models to evaluate the relationship between ALT and HIV/HBV co-infection, among HIV-infected women, controlling for ART regimen, CD4 count and visit. All individuals were included in the analysis regardless of the different numbers of follow-up visits. To show the change of ALT levels longitudinal line graphs were used. Predictions of ALT levels per visit were also plotted using margin plots.

Results

The study subjects comprised of 544 women of whom 5.6% were HIV/HBV co-infected. The age range of the study population was 16-45 years. Median age at enrolment was 26(IQR: 22-29). The median ALT enzyme level of HIV/HBV co-infected individuals was slightly higher at baseline (13 UI/L (10-16) vs 14 UI/L (11-18, p=0.10) and at the last follow-up (17UI/L (14-22) vs 19 UI/L (16-26, p=0.04) compared to HIV mono-infected counterparts. HIV/HBV co-infection women were 3.28 times (1.43-9.03 p= 0.01) more likely to have abnormal ALT, compared to their mono-HIV infected counterparts. Individuals that were initiated on Nelfinavar as first line ART were 3.22

times (1.85-5.59 p=0.001) more likely to have elevated ALT compared to those that were initiated on LPV/r based regimen. Moderately immune suppressed women (CD4 count of between 200 to 500 cells/dl) were 0.38 times less likely to have elevated ALT(0.15-1.00) while women who were severely immune suppressed had 3.51 times more likely to have abnormal ALT . Overall there was an increase in the level of ALT per each subsequent visit.

Conclusion

Individuals co-infected with HIV/HBV generally had higher levels of ALT compared to HIV mono-Infected individuals and this increased over time. The current study suggests that monitoring of ALT in patients co-infected with HIV/ HBV on ART should be performed regularly, and the caution should be taken when prescribing first line ART.

APPENDICES

Appendix 1: Ethics clearance certificate from the University of Witwatersrand Research Ethics Committee

Appendix 2: Malawi Ethics Approval

Appendix 3: HREC-90-06-Chasela Approval Letter 11-June-07

LIST OF ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
ALT	Alanine aminotransferase
ART	Antiretroviral Therapy
BAN	Breastfeeding Antiretroviral and Nutrition study
CDC	Centres for Disease Control
DNA	Deoxyribonucleic Acid
D4T	Stavudine
GLMM	Generalised linear mixed models
HAART	Highly Active Antiretroviral Therapy
HBV	Hepatitis B virus
HIV	Human Immunodeficiency Virus
IRIS	Immune Reconstitution Inflammatory Syndrome
HBeAg	Hepatitis B e antigen
HBeAb	Hepatitis B e antibody
HBsAb	Hepatitis B surface antibody
HBsAg	Hepatitis B surface antigen
LPV/r	Lopinavir and Rotinativir
MTCT	Mother to child transmission
NVP	Nevirapine
ZDV	Zidovudine
3TC	Lamivudine
SSA	Sub Saharan Africa

TABLE OF CONTENTS

DECLARATION ii
DEDICATION iii
ACKNOWLEDGEMENTS iv
ABSTRACT v
Background v
Methods v
Results v
Conclusion vi
APPENDICES vii
LIST OF ABBREVIATIONS viii
LIST OF TABLES xi
LIST OF FIGURES xii
1.0 INTRODUCTION 1
1.1 Background 1
1.1.1 Problem Statement 2
1.1.2 Justification for the Study 2
1.2 Literature Review 3
1.2.1 Introduction 3
1.2.2 HIV burden in Malawi 3
1.2.3 HBV Burden 3
1.2.4 Phases of HBV Infection 4
1.2.5 HIV/HBV co-Infection Prevalence 5
1.2.6 Effects of ART on the Liver 5
1.2.7 Alanine Amino Transferase as an Indicator of Liver Damage 6
1.2.8 Summary of Literature review 7
1.2.9 Study Objectives 7
2.0 METHODS 9
2.1 Introduction 9
2.2 Study Design and Description of the Secondary Study 9
2.3 Study Setting 9
2.4 The Primary Study (BAN Study) 9

2.5 Study Population and Sampling 10
2.6 Data Sources 10
2.7 Data Management and Analysis 10
2.7.1 Measurements of Exposure and Outcome 10
2.7.2 Data Management 11
2.7.3 Exploratory Analysis 12
2.7.4 Objective 1: Descriptive Analysis of Demographic Data 12
2.7.5 Objective 2: Comparison of the Level of ALT at Baseline and Last Follow-Up 12
2.7.6 Objective 3: Factors that Affect the Levels of ALT in the HIV/HBV co-infected Individuals 13
3.0 RESULTS 14
3.1 Introduction 14
3.2 Study Population 14
3.3 Baseline Characteristics 15
3.4 Clinical Laboratory Characteristics 15
3.4.1 Baseline Characteristics Stratified by HIV/HBV co-infection 17
3.5 Change of ALT over Time among HIV/HBV Co-Infected and HIV Mono-infected Women 17
3.6 Factors associated with changes in the levels of ALT 17
3.7 Factors associated with Elevated ALT 22
4.0 DISCUSSION 23
4.1 Introduction 23
4.1.1 Effect of HIV/HBV co-infection among women on ART 23
4.1.2 Change of ALT Levels per Visit 24
4.1.3 The effects of ART on the Levels of ALT 24
4.1.4 Nevirapine based Regimen 25
4.1.5 LPV/r based Regimen 25
4.1.6 Nelfinavir based regimen 25
4.1.7 CD4 cell count and levels of ALT 26
4.2 Limitations 26
4.3 Conclusion 27
5.0 REFERENCES I
6.0 APPENDECES V
6.1 Appendix 1: Wits Ethics Clearance Letter V
6.2 Appendix 2: Malawi Ethics Approval VI

6.3 Appendix 3: HREC-90-06-Chasela Approval Letter VII

LIST OF TABLES

Table 1: Baseline characteristics and ART regimens stratified by HIV/HBV co-infection
Table 2: Factors Associated with the changes in the levels of ALT
Table 3: Predictors of Elevated ALT

LIST OF FIGURES

Figure 1: Conceptual Diagram 14

Figure 2: Changes of ALT levels between HIV mono-infected and HIV/HBV co-infected individuals .....**Error! Bookmark not defined.**

Figure 3: Margin plot for Predicted ALT levels per visit for both groups ..... **Error! Bookmark not defined.**

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Of the 40 million people currently living with HIV/AIDS worldwide, 70% reside in sub-Saharan Africa (SSA) (1)2) . Malawi has a generalized HIV epidemic with a prevalence of 10.6% among adults (15-49 years old) as of 2010. In 2012 it was estimated that about 1.1 million out of 17.74 million people were living with HIV. AIDS still remain the leading cause of death amongst adults in Malawi, it affects the life expectancy. In contrast, the number of people living with active hepatitis B virus (HBV) infection worldwide is estimated to be at least six times higher than that of those infected with HIV(3). At least 275 million people worldwide are estimated to be chronic carriers of HBV, representing over 5% of the global population. HBV is endemic in sub-Saharan Africa (4), the general carrier rate in SSA's general population is from 5-20%

The prevalence of hepatitis B surface antigen (HBsAg) seropositivity among HIV infected individuals is ten times higher than in the general population (5). Due to shared modes of transmission, Co-infection with HIV and hepatitis B virus is common. HBV prevalence among HIV infected individuals varies with population studied nevertheless many studies have shown increased prevalence of HBV among HIV infected populations in SSA. It is estimated that 7.4% of HIV infected individuals are HBV infected. The natural progression of chronic HBV infection is modified in the presence of HIV co-infection (6).

The national governments and International donors have made commendable efforts in scaling up HIV treatment and care which resulted into a large scale roll out of ART(7). The large scale availability of ART has resulted in a decrease in the mortality and morbidity due to AIDS defining diseases. Following improved life expectancy non-Aids defining diseases are surfacing as a common cause of death among HIV infected individuals with liver disease as a regular cause of mortality. This proportionately high liver-related mortality is due to the accelerated course of hepatitis B in HIV-seropositive patients (8). Persistent HBeAg reactivity and high levels of HBV DNA in the blood have been associated with an accelerated progression of hepatitis B in HIV

co-infected persons. The outcomes of HIV/HBV co-infection includes increased liver-related morbidity and mortality(6), immune reconstitution to HBV in the setting of ART, increased hepatitis B viral replication and hepatotoxicity from antiretroviral drugs(8)(9)

Given the large scale ART roll out the burden of liver disease is expected to increase especially in HBV co-infected individuals. Knowledge of the pattern of ALT levels will inform the effect of HBV infection among individuals on lifelong ART. This study therefore will assess the changes of the levels of ALT to establish liver damage among HIV/HBV co-infected women on ART recruited in the Breast feeding Antiretroviral and Nutrition (BAN) study Malawi.

1.1.1 Problem Statement

Malawi implemented the new integrated ART/PMTCT guidelines in July 2011(10). The implementation included initiation of life long ART for all HIV infected pregnant and breastfeeding women irrespective of their WHO stage and CD4 cell count(10). Such a large scale up of ART in the country with high burden of HBV will lead to high risk of liver disease (11). The additive effects of HBV on the liver have been studied but there is little knowledge among women in Malawi.

Using the BAN study data we conducted a secondary data analysis to assess the liver damage using ALT as a proxy. The BAN study assessed the effect of ART in reducing MTCT of HIV. Women were followed for 48 weeks post-delivery with biomedical monitoring, in addition women were tested for HBV infection at Baseline.

1.1.2 Justification for the Study

With more people getting onto ART knowledge of the risks of ART on those with HBV or the extent of liver damage will help in deciding on choice of first line ART. For example if HBV is diagnosed before initiation of ART, caution will be taken in the prescription of first line ART for those patients who are HIV/ HBV co-infected given the high risk of liver damage among these individuals. Drugs such as abacavir, saquinaver, Nelfinavir, Lamivudine, Amprenavir and Indinavir may be prescribed as first line therapy because they are less toxic to the liver (12). Tenofovir and Emtricitabine may be included in the first line ART because they have dual activity against HIV and HBV (13). Close monitoring of ALT may help to understand the

patterns of the changes in the levels of ALT which will lead to early diagnosis of liver damage.

1.2 Literature Review

1.2.1 Introduction

It is estimated that over 280 million people worldwide are infected with HBV chronically (Positive hepatitis B surface antigen positive for over 6 months) while 40 million people are infected with HIV. Because of the shared way of transmission, HIV/HBV co-infection is common, and about 4 million people in the world are HIV/HBV co-infected. Studies have reported a relatively high prevalence of HBV in HIV infected populations of sub-Saharan Africa with more than 36% of the HIV positive individuals are carrying serum markers for HBV. HIV/HBV co-infection is a major public health problem which influences morbidity and mortality in sub-Saharan Africa. HBV prevalence among HIV infected individuals, is estimated at 4%-24%(9). The common modes of transmission are vertical horizontal and early childhood exposure.

1.2.2 HIV burden in Malawi

HIV prevalence in Malawi is among the highest in the world with 10.3% of the population living with HIV(3). Malawi contributes 4% of the total number of people living with HIV in SSA. In 2011 Malawi adopted option B+ plus which involved lifelong ART treatment for all breastfeeding mothers regardless of CD4 count or clinical stage(10). In 2012, the number of women on any antiretroviral during pregnancy expanded to 20,687 from 13,910 in 2011(10). This was a 49% increase in total antiretroviral coverage of known HIV positive pregnant women. With such a large ART scale up, the risk of liver disease is expected to be high especially among HBV co-infected individuals.

1.2.3 HBV Burden

Hepatitis B virus attacks the liver, and it causes acute and chronic liver disease. The virus can be transmitted through exposure to infected body fluids or blood. It is frequently spread from mother to child during birth (perinatal transmission) in endemic areas. Sexual transmission may occur, in unvaccinated men who have sex with men and heterosexual persons with multiple sex partners. Less than 5% of adult cases progress to chronic hepatitis B (6). In addition transmission of the virus may occur

through the reuse of skin piercing instruments i.e. Needles and syringes either in hospital settings or among individuals who inject drugs. It is estimated that 275 million people are chronically infected with hepatitis B (defined as hepatitis B surface antigen positive for at least 6 months). About 686 000 people die every year due to complications of hepatitis B, which includes cirrhosis. Hepatitis B prevalence is high in East Asia and sub-Saharan Africa, it estimated that about 5–10% of the adult population is chronically infected with hepatitis B virus in Africa and Asia(14).

1.2.4 Phases of HBV Infection

The individual course of HBV infection depends on the interaction between host immune system and the virus replication. The phases can be divided into four: immune tolerance, immune clearance, low or Non_replicative and reactivation.

1.2.4.1 Immune Tolerant

Patients have HBeAg with high serum levels of HBV DNA but normal or mildly elevated ALT and normal or minimal liver histological activity.

1.2.4.2 Immune Active Phase

After a period of HBe Ag positivity, which depends on the age at which infection was acquired, the immune tolerance is lost and patients may enter to immune active phase. This phase is characterised by fluctuating but decreasing HBV DNA levels and increased ALT and Histologic activity indicating immune mediated histologic damage.

1.2.4.3 Low or Non-Replicative Phase

This involves seroconversion from HBeAG to its antibody anti_HBe. This is preceded by decrease in serum HBV DNA to below detectable levels, and is referred to carrier inactive phase, liver disease is inactive with normal ALT. Inactive phase can last for decades or for life.

1.2.4.4 Reactivation Phase

In a few individuals who were initially positive for HBsAG receiving highly active antiretroviral therapy (HAART) for HIV infection, HBV-associated episodes of severe hepatitis have occurred and mostly were explained by HAART-induced immune reconstitution.

1.2.5 HIV/HBV co-Infection Prevalence

HIV accelerates the natural progression of HBV infection. The prior presence of HIV in HBV patients increases the risk of developing chronic HBV and prolonged ALT elevation. HBV/HIV co-infection slows and reduces the rate of HBeAg and HBsAg sero conversion, which results into higher prevalence of HBeAg-positive disease. There is a link between HIV and reactivation of HBV and increased HBV viral load, despite milder levels of serum ALT compared to HBV mono-infected individuals. Despite this liver damage progresses more rapidly shortening the period from HBV acquisition to Cirrhosis in co-infected individuals and also they have poor response to interferon and increased lamivudine resistance. Acute HBV may be accelerated in the presence of HIV infection, with a lower incidence of icteric illness and poor clearance of HBV(15). HIV increases the risk of cirrhosis and end-stage liver disease in HBV co-infection(16). Liver-related disease has emerged as the leading cause of non-HIV-related mortality in countries with large coverage of ART(2). Cohort studies have shown risk of liver-related mortality to be 2-3 times higher in HIV/HBV co-infected patients than in HIV-mono infected patients (14% vs 6%)(5)(6). HIV co-infection is associated with more frequent fluctuations of ALT, which result from immune reconstitution inflammatory syndrome (IRIS) owing to ART, interruption of HIV/HBV treatment, or the development of resistance to HIV/HBV treatment (9).

1.2.6 Effects of ART on the Liver

Listed below are the mechanisms by which ART can cause liver damage mitochondrial toxicity, Hypersensitivity reactions, IRIS, Direct drug toxicity or drug metabolism. IRIS is characterized by the worsening of preexisting infectious diseases due to rapid immune restoration in the setting of successful HIV RNA suppression. The syndrome generally manifests within the first two months of ART initiation and is accompanied by a precipitous decline in HIV RNA and rise in CD4 count. In patients with viral hepatitis, immune restoration can lead to a clinical hepatitis due to the immune response to the virus. There have been case reports of clinical flares of HBV in the setting of ART initiation, even with regimens including anti-HBV activity, and of rapidly progressive HCV-related cirrhosis associated with ART-related immune restoration Co-infection with HBV 2.74 (0.37-5.12). Other risk factors associated with ART-related liver injury include pre-existing advanced fibrosis, pre-treatment elevated ALT or AST, alcohol abuse, older age, female gender, first exposure to ART, and significant

increase in CD4 cell count after ART initiation, concomitant tuberculosis medications, and cocaine use.

While all antiretroviral drugs have some risk of hepatotoxicity, some are more implicated than others, and classes of drugs have characteristic patterns of injury. The non-nucleoside reverse transcriptase inhibitors (NNRTI's) typically cause either hypersensitivity reactions or direct drug toxicity and therefore have two peaks of onset: within days to weeks or several months after initiation. Nevirapine (NVP) is the NNRTI most associated with hepatotoxicity, though hypersensitivity reactions resulting in liver failure have been reported with the newer NNRTI etravirine. Efavirenz can also cause hepatotoxicity but does so less frequently than NVP or etravirine.

Ritonavir is known to be hepatoxic while saquinaver, Nelfinavir and Amprenavir and Indinavir have shown to be safer protease inhibitors (17). Lamivudine has been reported to be effective against both HIV and HBV replication, although it has been reported to be resistant to HIV/HBV co-infected individuals recently. Resistance to lamivudine was reported in 50% after 2 years and 90% after 4 years of therapy in a retrospective cohort study of HIV/HBV-co-infected persons(18).

Tenofovir has recently been reported to be active against both HIV and HBV. Tenofovir is a nucleotide reverse transcriptase inhibitor, and it has been shown to have potent in vitro activity against both wild-type and lamivudine-resistant HBV. In pilot studies, Tenofovir demonstrated anti-HBV activity in HIV/ HBV co-infected patients. HBeAg sero-conversion was observed in a quarter of the cases after 52 weeks of treatment.

1.2.7 Alanine Amino Transferase as an Indicator of Liver Damage

Alanine aminotransferase (ALT) is a liver enzyme used to measure the liver damage in resource limited settings. Elevated levels of ALT are associated with liver damage and mortality (7). Measurement of ALT is critical in the diagnosis and assessment of liver damage. ALT has a longer half-life compared to the other liver enzymes which makes it very useful in monitoring liver damage. ALT is measured in blood as part of Liver function tests. Hepatitis B virus infection is the common aetiology of elevated ALT values. HBV infection is frequently asymptomatic and is sometimes discovered because of an elevated ALT level identified upon routine blood test (8). Elevated levels

of ALT (>43 UI/L) are associated with progression of liver disease and development of morbidity. ALT is used to test liver disease and treatment initiation and predicting the progression of the infection in future (19)(20). Hepatitis is associated with elevated ALT levels which indicate liver damage, known to be prevalent in patients who are on ART. However, studies of Tenofovir treatment of a larger patient population and for a longer period are necessary to assess the extent and durability of HBV suppression with this drug.

1.2.8 Summary of Literature review

Based on the studies that have been conducted on hepatoxicity of ART on HIV patients(7,12,17), there is little knowledge on the comparison of the extent of liver damage in patients who are HIV/HBV co-infected in Malawi. The results of this study will contribute towards filling this gap and in turn the management HIV/HBV patients.

1.2.8.1 Research Question

Does HIV/HBV co-infection affect the changes in levels of ALT among HIV/HBV co-infected compared to HIV mono infected among women on ART enrolled in the BAN study from 2004 to 2009 in Lilongwe Malawi (BAN study)?

1.2.9 Study Objectives

1.2.9.1 Objectives

1. Describe the social-demographic characteristics of HIV/HBV co-infected and HIV mono-infected women on ART enrolled in the BAN study from March 2004, to February 2009 in Lilongwe Malawi.
2. Compare the changes in the levels of ALT between HIV/HBV co-infected and HIV-mono infected women on ART enrolled in the BAN study from March 2004 to February 2009 in Lilongwe Malawi.
3. Identify risk factors associated with changes in ALT levels among HIV/HBV co-infected and HIV-mono infected women on ART enrolled in the BAN study from March 2004 to February 2009 in Lilongwe Malawi.

CHAPTER TWO

2.0 METHODS

2.1 Introduction

This chapter describes the methods used in the study and procedures that were employed to ensure the validity of the results. It includes the design of the study, a description of the study population, data collection and study variables. The chapter ends with details of data management carried out, the statistical methods used, the diagnostic test used to measure validity of the findings, including tests for confounding and interaction.

2.2 Study Design and Description of the Secondary Study

This is secondary data analysis of the Breast Feeding, ART and Nutritional (BAN) study in Malawi. In this analysis the ALT profiles over time were compared among 31 HIV/HBV co-infected and 513 HIV mono-infected women randomised into maternal ART arm of the BAN study. The subjects were followed over 20 follow ups for a period of 48weeks. ALT was used as continuous variable to track the mean change of the levels per follow-up, and was also used as categorical variable with categories normal; <2.5 times the upper limit of normal and abnormal; >2.5 times of the upper limit of normal.

2.3 Study Setting

The study participants were recruited from Bwaila antenatal clinic in Lilongwe Malawi. Samples were stored at University of North Carolina repository project Laboratory in Lilongwe, Malawi and there after shipped to Atlanta. Specimens were tested for Hepatitis virus at CDC Laboratory in Atlanta, Georgia, United states of America..

2.4 The Primary Study (BAN Study)

The BAN study was a 2 by 3 factorial randomised clinical trial conducted in Lilongwe Malawi. At delivery, women and their new-born were randomized to one of three, 28-week ART intervention arm with drugs given to the mother, infant, or neither—for prevention of HIV transmission during breastfeeding. The maternal ART regimen comprised Zidovudine, Lamivudine, and either NVP, Nelfinavir (NFV) or Lopinavir/ritonavir (LPV/r). Alanine-aminotransferase (ALT) was determined

antenatally, at delivery and 2, 4, 6, 8, 12, 18, 20, 24, 36 and 48 weeks postpartum. Details of the design are published elsewhere (21).

2.5 Study Population and Sampling

The proposed study utilized the data of 544 HIV positive women enrolled into BAN study from March 2004 to February 2009 at Bwaila antenatal clinic Lilongwe Malawi. This sample is based on 36% of women who were randomised into maternal ARV arm of the BAN study.

2.6 Data Sources

The data for this study were primarily from the BAN study conducted at Bwaila hospital Lilongwe Malawi. The socio-demographics information including age was collected using standard questionnaire. CD4 count were measured as part of
full blood count, while ALT was measured as part of Liver function tests. Information on ART is included in the dataset. Hepatitis B diagnosis was done retrospectively at CDC Laboratory in Atlanta in 2008 when a sub study of prevalence of hepatitis B and transmission was conducted (22)

2.7 Data Management and Analysis

2.7.1 Measurements of Exposure and Outcome

2.7.1.1 Exposure variables

HBV infection (categorical) was measured in a sub study of prevalence of HBV conducted in 2008. The stored plasma was shipped from -80^{0}C storage in Lilongwe to repository at CDC Atlanta and tested at the CDCs division of viral Hepatitis. The plasma specimen from second and third trimester of pregnancy at pre-randomisation eligibility screening were tested for total antibody to hepatitis B core antigen(anti-HBc) and those anti-HBc-positive were assayed for Hepatitis surface antigen (HBsAg) and those HBsAg-positive were tested for hepatitis e antigen. Specimen found HBsAg positive underwent HBV DNA quantification (22). Based on HBsAg positivity the prevalence of HBV in this group of women was 5.6%.

2.7.1.2 Study outcome

ALT: plasma alanine aminotransferase (ALT) level was measured compared to the upper limit of normal. It was analysed as both a continuous and categorical variable.

ALT level of < 43IU/L is considered normal and >43 IU/L was abnormal or Transaminitis.

2.7.1.3 Potential Confounders

i. Socio-Demographic

- Age: Age in years at time of enrolment

ii. Biological

- Regimen of ART: The first line ART was ZDV, 3TC and NVP (Nevirapine based regimen), individuals who developed toxicity to ZDV were switched to Stavudine. On February 7, 2005, Nevirapine was discontinued as part of maternal HAART and was replaced with Nelfinavir. After February 7 2005 ZDV or DT4, 3TC and Nelfinavir (Nelfinavir based) was the first line ART. On January 25, 2006 Nelfinavir was replaced by LPV/r and the first line regimen was ZDV or DT4, 3TC and LPV/r (LPV/r based). These changes were made for safety, availability and potency.
- CD4 count: number of cells/ μl was categorised into Immune suppression groups according to CDC guidelines into
    1. No immune suppression = CD4 count >=500 cells/ μl
    2. Moderate Immune suppressed= >=200 -500 cells/ul
    3. Severe immune suppression = <200 cells/ μl
- Hepatitis B surface antigen was measured using the Vitros Chemiluminescence Immunoassay (Ortho Clinical Diagnostics, Rochester, NY)

2.7.2 Data Management

The data from the original study was already in stata format. The data was cleaned and checked for duplicates and missing data. Renaming, replacement and recoding of relevant variables was done. All variables not needed for the current study were dropped. Continuous data that is required in categorical form were categorised. Data in wide format was converted into long format for analysis. Exploratory analysis to summarize the main characteristics of the data and to identify outliers, trends and data patterns was done using graphical methods like box plots and histograms. Normal

probability plots and histograms were used to determine if data has a normal distribution for all continuous variables.

2.7.3 Exploratory Analysis

Exploratory analysis was done to summarize the data and identify missing data trends, patterns and outliers. Histograms, skewness test and Shapiro wilk test were used to check if the continuous data is normally distributed. Non-parametric test was employed on all skewed data. Exploratory analysis of data was achieved using commands such as misstable, summarise in Stata, to view the patterns of missingness across the follow-ups.

2.7.4 Objective 1: Descriptive Analysis of Demographic Data

Descriptive analysis included generating a small dataset that included the first follow-up and the last follow-up in stata. This was done to get a smaller dataset for descriptive analysis. All participants had first and last follow-ups regardless of the number of visits. All 544 participants were included in the descriptive analysis. Participants were categorised as HIV infected or HIV/HBV co-infected. The baseline characteristics for each group were summarised using median and interquartile ranges for all continuous skewed data, and simple proportions with confidence intervals were determined using exact methods for categorical variables. Continuous variables were compared using Wilcoxon rank sum test and Sign test between the two groups. Two way comparisons of frequencies of categorical data were done using Pearson's chi squared-tests where the expected value is greater than or equal to 5 and Fischer's exact where the expected cell value is less than 5. A p-value of <0.05 was considered as significant in all analysis.

2.7.5 Objective 2: Comparison of the Level of ALT at Baseline and Last Follow-Up

A data set for 2 follow-ups was generated in stata, which included the first follow-up and the last follow-up to compare the change in ALT, from baseline to last follow-up. Wilcoxon rank sum was also used to compare ALT levels between the groups at baseline and at the last follow-up of each individual

2.7.6 Objective 3: Factors that Affect the Levels of ALT in the HIV/HBV co-infected Individuals

All participants were included for the analysis even those who did not have all the follow ups. Generalised Linear Mixed Models was appropriate because it allows unbalanced or missing observations within-subject, unbalanced time intervals and allows various within-subject covariance structures. It also allows time to be treated as categorical or continuous. Univariate analysis GLMM was done to identify the variables to be adjusted for in the multivariate analysis. All variables with significant level below 0.05 were included in the GLMM model. GLMM analysis was used to estimate the effects of HBV on the levels of ALT over time. Models were adjusted for ART used (Nevirapine, Combivar, Nelfivar, stavudine LPV/r) and Visit number as determined in the univariate analysis. Wald statistic was used to select the more parsimonious model, the model with low Wald chi-square statistic was considered as the more parsimonious model. Backward elimination was used to build a parsimonious model.

All statistical tests were two sided and *P* –value <.05 was regarded as statistically significant. The results are reported as presented in the tables and graphs to meet the study's objectives.

Limitations

The BAN study was designed to investigate the effect of ART in prevention of mother–to-child transmission of HIV-1 during Breastfeeding not specifically to answer this secondary research question. Pregnant women recruited into BAN study were not a true representative of the general women population and this may affect the extrapolation ability of the study findings. Information on alcohol consumption and use of other medication is not available, these can be potential confounders. Among other factors liver damage can be caused by alcohol consumption and use of medication i.e. contraceptives , aflatoxins in some, and anti-Tuberculosis drugs. If this information was available we could adjust in the analysis.

CHAPTER THREE

3.0 RESULTS

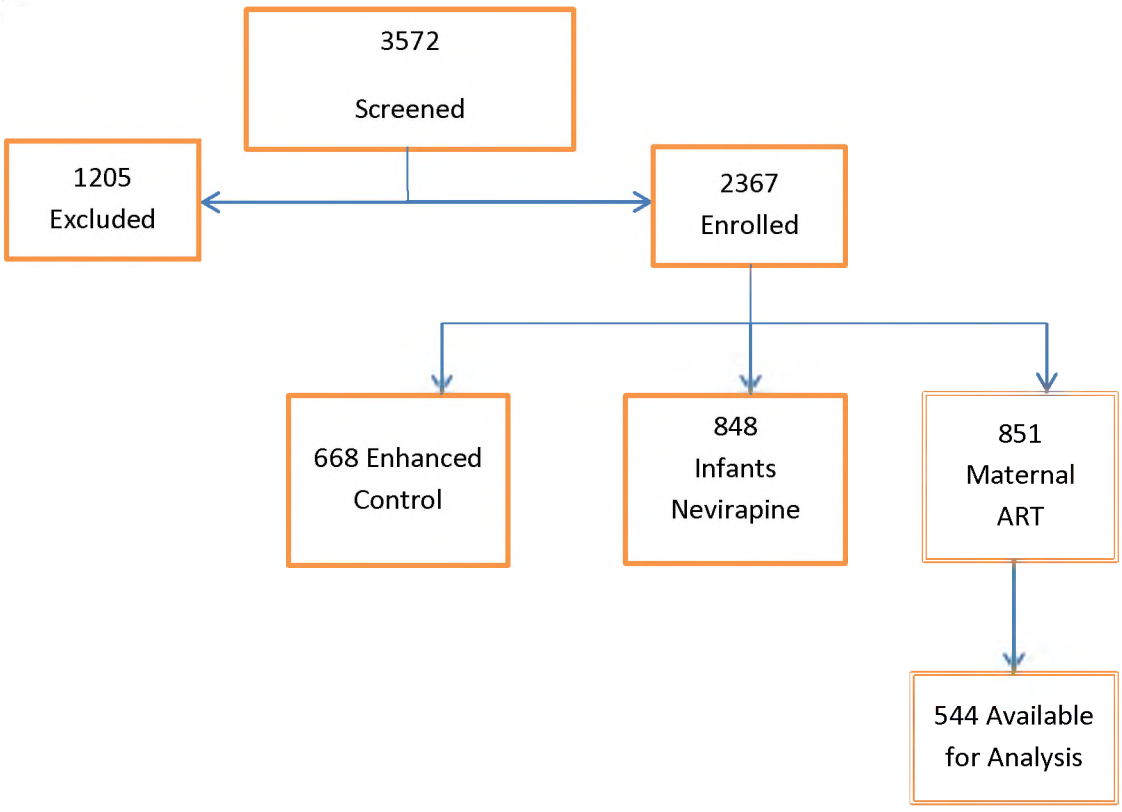
3.1 Introduction

The results are structured according to specific objectives of this study and also show the results of the analysis that was done to explore bias and control for confounding.

3.2 Study Population

The BAN study screened 3572 antiretroviral-naive, HIV-infected pregnant women attending 3 antenatal clinics in Lilongwe and enrolled 2367 women who met antenatal and postnatal eligibility criteria between March 2004 and February 2009. A total of 2367 mothers and child pairs were included in the BAN study: 851 were randomised to maternal ART, 848 to infant NVP, and 668 to the enhanced control. The enhanced control arm had fewer women, because randomisation to the control arm was discontinued after March 2008 following the Data Safety and Monitoring Board (DSMB) recommendations. Of the 851 women randomised to the maternal art arm 544 were made available for this analysis.

Figure 1: Consort Diagram



3.3 Baseline Characteristics

A total of 544 women randomised into the maternal ART arm of the BAN study were analysed at baseline, of which 39 (7.23%) women were initiated on Nevirapine based regimen as their first line regimen, due to change in the FDA guidelines the first line ART regimen changed to Nelfinavar based regimen and 107 (20.11%) of the women were initiated on Nelfinavar based regimen as their first line regimen. Two years later FDA guidelines changed and Nelfinavir was replaced with Lopinavir/Ritonavir as first line regimen and 389 (72.56) women were initiated on LPV/r based regimen. Median age at enrolment was 26 (IQR:22-29).,

3.4 Clinical Laboratory Characteristics

Of 544 women analysed at baseline 31 (5.6%) were HIV/HBV co-infected. At baseline median ALT was 13(10.5-16), and median CD4 count was 435.5 cells/µl (331-565.5), 2 (0.37%) of the women had severe immune suppression (CD4 less than 200 (cells/µl) and majority (62.87%) of the women were moderately immune suppressed and 36.76% had CD4 cell count above 500 cells/ul.

Table 1: Baseline characteristics and ART regimens stratified by HIV/HBV co-infection

Characteristics	Overall (Median/ Percentage)	HIV/HBV(31)	HIV(513)	p-value
Age (Median)	26 (22-29)	24(22-29)	26(22-29)	0.29¥
ALT (Median)	13 (10.5-16)	14(11-18)	13(10-16)	0.09†
ART regimen (%)				
AZT/DT4/3TC/ LPV/r	386 (72.56)	18 (60.00)	371 (73.31)	
AZT/DT4/3TC/ Nelfinavar	107 (20.11)	11 (36.67)	96 (19.12)	0.70†
AZT/DT4/3TC/Nevirapine	39 (7.23)	1 (3.33)	38 (7.57)	
Absolute CD4 (cells/µl)	435.5 (331-565.5)	442 (367-558)	435(327-566)	0.42
Absolute CD4 Count				
>500 cells/ µl)	200 (36.76)	13 (41.94)	187 (58.06)	0.62
>200 - <500 cells/ µl	342 (62.87)	18 (58.06)	324 (63.16)	
<200 cells/ µl	2 (0.37)	0	2 (0.39)	

Fischer's exact p-† value for binary variables.

¥ Wilcoxon rank sum test for skewed variables

* Variable significant at the 5% level of significance

3.4.1 Baseline Characteristics Stratified by HIV/HBV co-infection

Most baseline characteristics were similar between the HIV/HBV co-infected and HIV mono-infected women (Table1). However those co-infected were likely to have slightly elevated ALT compared to HIV mono-infected (p-value=0.10).The median levels of ALT were significantly higher 14 IU/L (11-18) vs. 13 IU/L (10-16)in HIV/HBV co-infected patients (p, 0.09).Comparing ALT levels at baseline and the last follow-up the results showed a significant difference (p, <0.001).Median ALT level was 14 IU/L (10-16) compared to 19 IU/L (16-26) of the last follow-up

3.5 Change of ALT over Time among HIV/HBV Co-Infected and HIV Mono-infected Women

To check the changes of ALT levels over time, margins of the final model with an interaction term visit and HIV/HBV co-infection were calculated and the predicted changes of ALT levels were plotted. Figure (2) shows predicted levels of ALT at each visit. Predicted levels of ALT consistently higher among HIV/HBV co-infected women through the visits. Figure (3) shows the changes in the levels of ALT over the follow up period. HIV/HBV co-infected women had on average higher median ALT levels. At baseline the levels were almost similar, but after four weeks into the study co-infected women started experiencing ALT elevation, and it picked at 8 weeks and then started going down to normal levels at 36 weeks.

3.6 Factors associated with changes in the levels of ALT

Table (2 & 3) shows predictors of ALT abnormality. Being HIV/HBV co-infected was associated with increased levels of ALT by 6.30 IU/L (1.87-7.47 p= 0.002). Women that were initiated on Nelfinavar based regimen had on average increased levels of the ALT throughout the study period ALT by 2.74 IU/L (0.37-5.12 p=0.02) Although not significant individuals on Nevirapine based regimen had increased levels of ALT compared to their counterparts who were initiated on LPV/r based regimen. In addition women who were severely immune suppressed had increased ALT 11.95 (P=0.03). The results also shows an increase in average levels of ALT at each subsequent visit. Each additional visit was associated with an increase in ALT level of 0.63 (P=0.0001).

Figure 2: Margin plot for Predicted ALT levels per visit for both groups

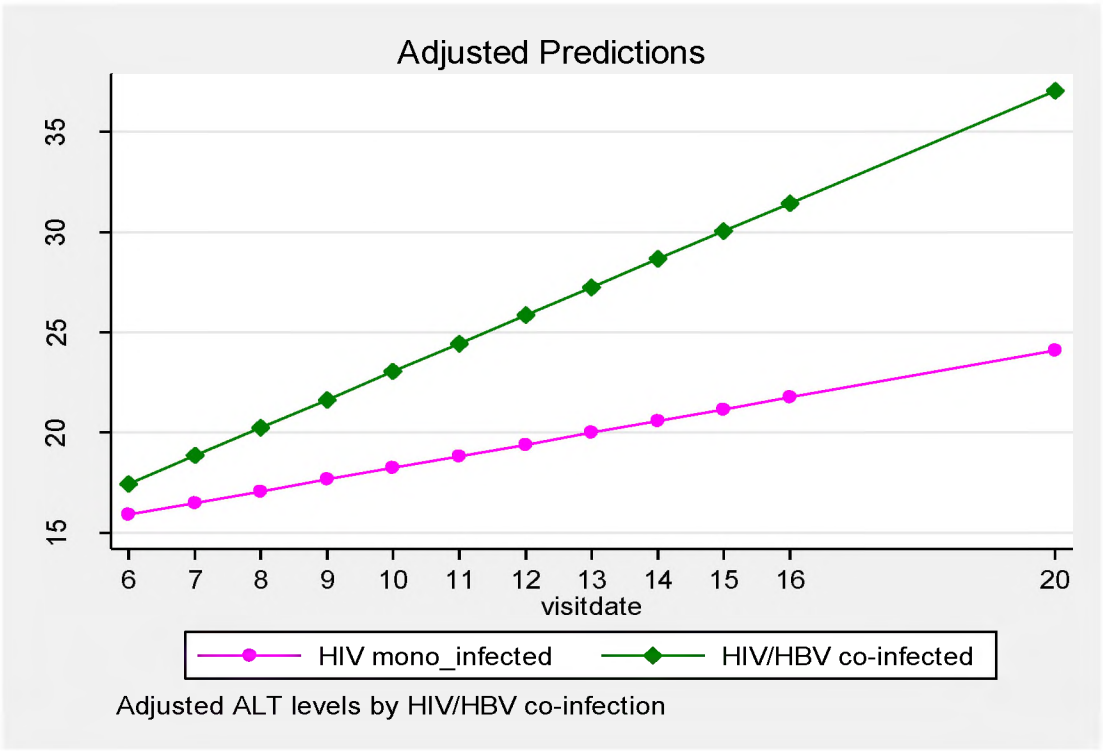


Figure 3: Profile plot changes of levels of ALT stratified by HIV/HBV co-infection

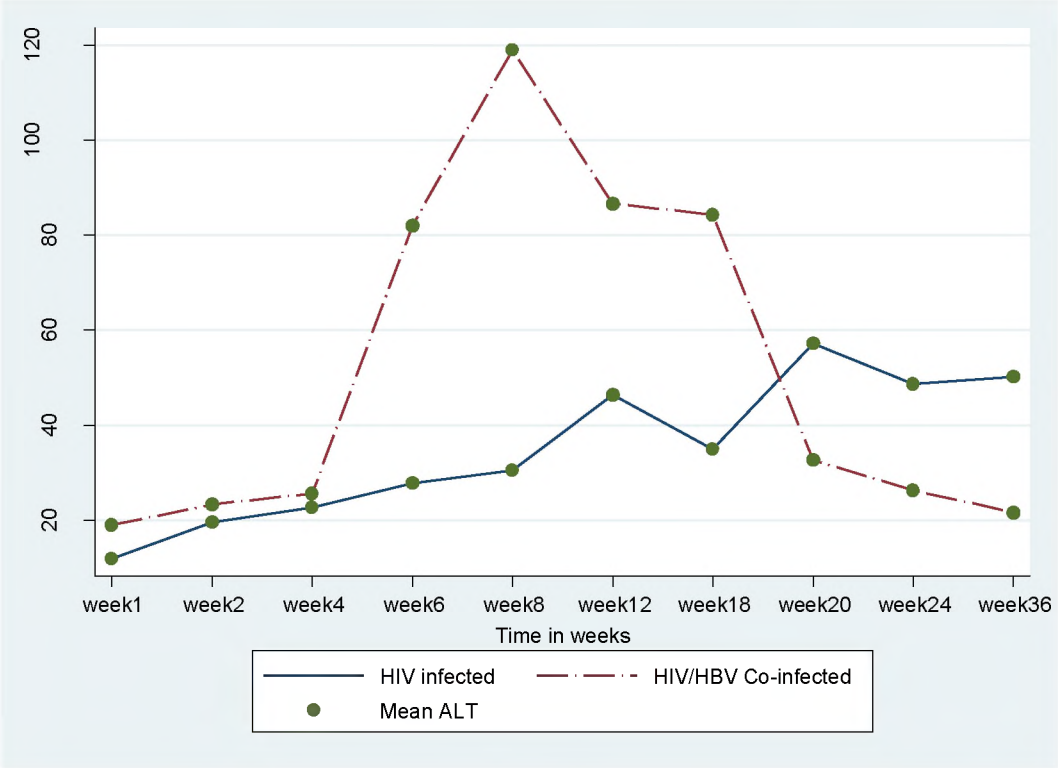


Table 2: Factors Associated with the changes in the levels of ALT

Variable	Un-adjusted Coefficient	P_value	Adjusted Coefficient	P_value
HIV+/HBV -	Ref			
HIV+/HBV+	6.37 (1.87-7.47)	0.01	6.78 (1.84-10.48)	0.05
Age	-0.0001(-0.00001-1.27)	0.10		
ART regimen				
AZT/DT4/3TC/ Kaletra	Ref			
AZT/DT4/3TC/ Nelfinavar	2.74 (0.37-5.12)	0.02	-0.53 (-4.37-3.31)	0.79
AZT/DT4/3TC/Nevirapine	3.78 (-0.778.340	0.10	0.90 (-6.32-8.13)	0.81
Absolute CD4 Count				
>500 cells/ µl)	Ref		Ref	
>200 - <500 cells/ µl	-2.67 (-5.70.	0.09	-0.81 (-3.99-2.38)	0.62
<200 cells/ µl	11.95 (0.92-22.99)	0.03	11.32 (0.29-22.34	0.04

Visit-number	0.63	<0.01	0.63 (1.06-1.12)	<0.001

Table 3: Predictors of Elevated ALT

Variable	Un-Adjusted OR (95% CI)	P-value	Adjusted-OR (95% CI)	P-value
HIV+/HBV-	Ref			
HIV+/HBV+	3.28 (1.43-9.03)	0.01	3.27 (1.48-7.09)	0.01
Age	1.02 (0.96-1.07)	0.43		
ART regimen				
AZT/DT4/3TC/ Kaletra			Ref	
AZT/DT4/3TC/ Nelfinavar	3.22 (1.85-5.59)	<0.001	0.85 (0.5-1.04)	0.54
AZT/DT4/3TC/Nevirapine	2.69 (1.04-7.01)	0.04	1.9 (0.13-4.8)	0.13
Absolute CD4 Count				
>500 cells/ µl)	Ref		Ref	
>200 - <500 cells/ µl	0.38 (0.15-1.00)	0.05	1.24 (0.83-1.87)	0.28
<200 cells/ µl	3.51 (0.75-16.27)	0.12	1.03 (0.70-6.49)	0.18
Visit-number	1.19 (1.11-1.27)	<0.001	1.12 (1.15-1.27)	<0.001

3.7 Factors associated with Elevated ALT

Transaminitis is defined as elevated ALT levels above 43 IU/L. to check for the risk factors of Elevated ALT between HIV/HBV co-infected and HIV mono-infected individuals ALT was categorised into high (≥43 IU/L) and normal (<.43 IU/L). Table (3) shows results of multiple regression analysis with categorical ALT as dependent variable. HIV/HBV co-infected women were 3.28 (1.43-9.03 p= 0.01) times more likely to have higher ALT (Transaminitis), compared to their HIV mono-infected counterparts. Individuals that were initiated on Nelfinavar as first line ART were 3.22 (1.85-5.59 p=0.001) more likely to have transaminitis compared to those that were initiated on LPV/r based regimen. Moderately immune suppressed women (CD4 count of between 200 to 500 cells/dl) were 0.38 times less likely to have transaminitis (0.15-1.00) while women who were severely immune suppressed were 3.51 times more likely to have transaminitis. Overall there was an increase in the level of ALT per each subsequent visit. For each subsequent visit, there was increase in average ALT (0.506; p-value 0.0001).

CHAPTER FOUR

4.0 DISCUSSION

4.1 Introduction

This chapter discusses the findings for the study outcome. We will discuss the factors that are associated with our outcome, starting with the main exposure of the study which is HIV/HBV co-infection. Relationships between the findings in our study compared to findings from other studies are explored and possible reasons for our findings are also discussed. The chapter also gives an overview of the relevance of the findings with regards to HIV, HBV and Liver disease and also highlights the limitations of the study.

4.1.1 Effect of HIV/HBV co-infection among women on ART

This study assessed changes of the levels of ALT to determine the effect of HIV/HBV co-infection on liver disease using ALT as proxy, among women on ART enrolled in the Breast feeding Antiretroviral and Nutrition (BAN) study in Malawi.

Our results show that HIV/HBV co-infection had an impact on the levels of ALT. At baseline there were no clear difference in the levels of ALT among HIV/HBV co-infected individuals and HIV infected. Higher Median serum ALT enzyme levels, were reported among HIV/HBV co-infected women from 4 weeks after ART initiation picked at week 8 up to 5 times upper limit of normal and started going down to normal levels (Figure 2). In addition Individuals that were initiated on Nevirapine and Nelfinavar based regimens had increased chances of developing transaminitis compared to those that were initiated on LPV/r based regimen. Immune suppression at baseline was also significantly associated with higher levels of ALT. The findings of this study are consistent with previous study conducted among ART naïve population in Nigeria B.Goni et al. (23). The study showed that HIV/HBV co-infected patients had an Increased median ALT levels i.e., 42.12 IU/l vs. 27.86 IU/l, (P = 0.038). Aluwami H O, reported high levels of ALT among HIV/HBV co-infected individuals(23).

Increased risk of liver disease in HIV/HBV co-infected women on ART can be explained by Immune reconstitution after ART initiation, which is the immune mediated destruction of the liver cells that are infected by the HBV virus after ART initiation. The

individual course of HBV infection depends on the interaction between host immune system and the virus replication. The normal HBV progression in individuals who are not HIV infected can be divided into four phases, immune tolerance, immune clearance, low or Non_replicative and reactivation. Immune tolerance phase patients have High levels of HBV DNA, with mildly elevated ALT and minimal histological activity. After a period of immune tolerant phase depending on the age individuals may enter into immune active phase where patients experience fluctuating HBV DNA levels and increased ALT and liver histologic activity which indicates immune mediated histologic damage.90 % of individuals proceed to inactive where HBV DNA is decrease to below detectable levels. This phase can last for life if the patient's immunity is not compromised. In immune compromised individuals i.e. HIV infected individuals can experience HBV infection reactivated caused by immune reconstitution by ART.

4.1.2 Change of ALT Levels per Visit

On average each subsequent visit was associated with an increase in the level of ALT the increase was much higher in individuals Co-infected with HIV/HBV.Price J te.al recorded accelerated progression of liver disease in the presence of HIV, and was attributed to lower rates of spontaneous clearance of HBeAg, increased HBV replication, and higher rate of loss of anti-HB and reactivation of HBV (24). They indicated that co-infected individuals also experienced an increased progression to cirrhosis and higher liver related mortality. In addition, HIV/HBV co-infected individuals experienced frequent hepatic flares of ALT over the 48 weeks of follow-ups. These flares can be attributed to immune reconstitution due to a rapid increase in cytotoxic T cells, leading to immune-mediated destruction of HBV-infected hepatocytes (16). These findings are in line with a study by Hans H et al (25), which reported 26(3.4%) patients experienced severe hepatoxicity out of the 755 patients who were receiving ART.

4.1.3 The effects of ART on the Levels of ALT

Liver toxicity is one of the most common adverse events associated with ART. The clinical presentation can range from mild asymptomatic increases in serum transaminases to liver failure(26). In retrospective studies, the incidence of ART-

related severe hepatotoxicity has been estimated to be 10%, and life-threatening events occur at a rate of 2.6 per 100 person years(24). While all antiretroviral drugs have some risk of hepatotoxicity, some are more implicated than others.

4.1.4 Nevirapine based Regimen

In agreement with other studies this study showed that Nevirapine is associated with increased ALT levels. Individuals on Nevirapine had increased levels of ALT compared to their to individuals who were Kaletra based regimen. The potential of increased liver toxicity of a Nevirapine based regime compared to other regimens has been previously described by Paul, E (27)(28). Paul reported toxicity in 1 (5%) of 21 subjects on Nelfinavir based regimen and 5 (29%) of 17 subjects randomized to Nevirapine. Among the individuals randomised to the Nevirapine group, 1 subject developed hepatic failure and died, and another developed Stevens-Johnson syndrome. Esteban,E, (29) reported 12 percent incidence of Hepatoxicity among ART naïve patients receiving Nevirapine based regimen. In addition Nevirapine has been implicated to cause hypersensitivity which includes skin rash, eosinophilia and Hepatitis (23).The findings of this study are consistent with literature. Antonio R et el. also reported increased levels of ALT among patients on Nevirapine (30).

4.1.5 LPV/r based Regimen

LPV/r was associated with decreased ALT level of -2.76 IU/L (p. 0.013) in the current study. In contrast Sulkowski et al reported Lopinavir/Rotinativir to be toxic to the liver. Sulkowski, et al. recorded severe hepatotoxicity defined as elevated transaminases more than 5 times normal in 27.3% of patients on LPV/R treatment. These conflicting findings can be attributed to change of first line regimen in BAN study. In June 2005 BAN study changed the first line drugs from Nevirapine to Lopinavir/Rotinativir due to FDA recommendations (25). Not all individuals were initiated on LPV/r at baseline. Individuals who were enrolled before June 2005 were initiated on Nevirapine as a first line drug.

4.1.6 Nelfinavir based regimen

Nelfinavir has been recorded to cause low rates of liver toxicity, Mira, J(31) reported low incidence of severe hepatoxicity among patients on Nelfinavir based regimen

compared to Nevirapine. A 4268 patient's prospective and retrospective clinical trial and a prospective cohort study reported Nelfinavir to be associated with the lower rates of severe hepatotoxicity. The low rate of severe hepatotoxicity for Nelfinavir was reported even among participants that were co-infected with hepatitis viruses(32). This study shows Nelfinavir based regimen to be associated with increased levels of ALT, these findings are not consistent with other studies. These contrasting findings can be explained by the change in first line regimen the BAN study. First line ART regimen was switched from Nevirapine based regime to Nelfinavar based and then to LPV/r in a space of one year due to safety, availability and potency. So the high levels may not explicitly be attributed to Nelfinavir.

4.1.7 CD4 cell count and levels of ALT

Low CD4 cell count (<200 cells/ul) was associated with high levels of ALT in the current study. The effect of low CD4 count on liver disease has been extensively studied and there are conflicting findings on the effect of low CD4 cell count on the liver damage. H.O Olawumi et el reported increased rates of hepatotoxicity among patients with CD4 cell count of less than 200 cells/ul (23) among ART naïve population in Nigeria. P. J peters reported no association between Low CD4 cell count and hepatotoxicity among women on Nevirapine-based ART(33). These contradicting findings may be explained by understanding the association between Low CD4 cell count and Liver disease. Cassidy et el reported low CD4 cell count in patients with liver cirrhosis but HIV negative. He further reported sequestration of peripheral blood cells by the spleen in patients with liver disease , which can lead to low absolute CD4 cell count, but with normal CD4 percentages(26).

4.2 Limitations

The BAN study was designed to investigate the effect of ART in prevention of mother–to-child transmission of HIV-1 during Breastfeeding not specifically to answer this secondary research question. Pregnant women recruited into BAN study were not a true representative of the general women population this may affect the extrapolation ability of this study findings. Information on alcohol consumption and use of other medication is not available, these can be potential confounders. Among other factors liver damage can be caused by alcohol consumption and use of other medication i.e.

contraceptives, and anti-Tuberculosis drugs. If this information was available we could adjust in the analysis.

4.3 Conclusion

In conclusion our study shows that individuals co-infected with HIV/HBV are at a high risk of liver disease. Our study further strengthen the need for screening of all HIV positive patients for HBV before initiating treatment and also caution to be taken when choosing the first line ART for individuals co-infected with HIV/HBV since they are at very high risk of liver damage.

5.0 REFERENCES

1. Government of Malawi. Global AIDS Response Progress Report (GARPR): Malawi Progress Report for 2013. 2014;(March):1–45.

2. Mphahlele MJ. Impact of HIV co-infection on hepatitis B prevention and control : a view from sub-Saharan Africa. 2008;23(1):14–8.

3. Unaids. GLOBAL REPORT: UNAIDS report on the global AIDS epidemic 2013. Unaids. 2013. 198 p.

4. Review T, Thio C. Refl ection and Reaction HIV and hepatitis B co-infection in Africa. 2008;8(April).

5. Rj B. Hepatitis B virus and human immunodeficiency virus co-infection in sub-Saharan Africa : a call for further investigation. 2005;201–13.

6. Article S, Article S. Antiretroviral Therapy and HIV/Hepatitis B Virus Coinfection. Clin Infect Dis. 2004;38(Suppl 2):98–103.

7. Nagu TJ, Kanyangarara M, Hawkins C, Hertmark E, Chalamila G, Spiegelman D, et al. Elevated alanine aminotransferase in antiretroviral-naïve HIV-infected African patients: Magnitude and risk factors. HIV Med. 2012;13(9):541–8.

8. Guidelines for Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents.

9. Barth RE, Huijgen Q, Taljaard J, Hoepelman AIM. International Journal of Infectious Diseases Hepatitis B / C and HIV in sub-Saharan Africa : an association between highly prevalent infectious diseases . A systematic review and meta-analysis. Int J Infect Dis. 2010;14(12):e1024–31.

10. Considerations K. Malawi ' s Option B + HIV in Malawi.

11. Ahmed SD, Cuevas LE, Brabin BJ, Kazembe P, Broadhead R, Verhoeff FH, et al. Seroprevalence of hepatitis B and C and HIV in Malawian pregnant women. J Infect. 1998;37(3):248–51.

12. Hoffmann CJ, Charalambous S, Thio CL, Martin DJ, Pemba L, Fielding KL, et al. Hepatotoxicity in an African antiretroviral therapy cohort: the effect of

tuberculosis and hepatitis B. AIDS. 2007;21(10):1301–8.

13. Crum-Cianflone N, Dilay A, Collins G, Asher D, Campin R, Medina S, et al. Nonalcoholic fatty liver disease among HIV-infected persons. J Acquir Immune Defic Syndr. 2009;50(5):464–73.

14. Manegold C, Hannoun C, Wywiol A, Dietrich M, Polywka S, Chiwakata CB, et al. Reactivation of hepatitis B virus replication accompanied by acute hepatitis in patients receiving highly active antiretroviral therapy. Clin Infect Dis. 2001;32(1):144–8.

15. Dennis MK, Field AS, Burai R, Ramesh C, Whitney K, Bologa CG, et al. NIH Public Access. 2012;127(1):358–66.

16. Fix OK, Locarnini SA PM. Virology and Clinical Management of Hepatitis B and HIV Coinfection. Physicians Noteb. 2007;11(4):20–7.

17. Olczak A, Gra??bczewska E, Karwowska K. Hepatotoxicity of antiretroviral therapy. Gastroenterol Pol. 2009;16(4):301–3.

18. Recommendations K, Iv S, Vii S, Viii S, Hiv M, Viii S. What's New and Key Recommendations — August 2013 Update. 2013;(August):1–33.

19. Qu HQ, Li Q, Grove ML, Lu Y, Pan JJ, Rentfro AR, et al. Population-based Risk Factors for Elevated Alanine Aminotransferase in a South Texas Mexican-American Population. Arch Med Res. 2012;43(6):482–8.

20. Ruhl CE, Everhart JE. Elevated serum alanine aminotransferase and gamma-glutamyltransferase and mortality in the United States population. Gastroenterology. 2009;136(2):477–485.e11.

21. Chasela CS, Hudgens MG, Jamieson DJ, Kayira D, Hosseinipour MC, Kourtis AP, et al. Maternal or infant antiretroviral drugs to reduce HIV-1 transmission. N Engl J Med. 2010;362(24):2271–81.

22. Chasela CS, Kourtis AP, Wall P, Drobeniuc J, King CC, Thai H, et al. Hepatitis B virus infection among HIV-infected pregnant women in Malawi and transmission to infants. J Hepatol. 2014;60(3):508–14.

23. Olawumi HO, Olanrewaju DO, Shittu AO, Durotoye IA, Akande AA, Nyamngee A. Effect of hepatitis-B virus co-infection on CD4 cell count and liver function of HIV infected patients. Ghana Med J. 2014;48(2):96–100.

24. Price JC, Thio CL. Liver Disease in the HIV-Infected Individual. Clin Gastroenterol Hepatol [Internet]. 2011;8(12):1002–12. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2997131/

25. Hirsch HH, Kaufmann G, Sendi P, Battegay M. Immune Reconstitution in HIV-Infected Patients. 2004;38:1159–66.

26. Claassen CW, Diener-West M, Mehta SH, Thomas DL, Kirk GD. Discordance between CD4 + T-lymphocyte counts and percentages in HIV-infected persons with liver fibrosis. Clin Infect Dis. 2012;54(12):1806–13.

27. Paul ME, Foca M, Gandia J, Huang S. Maternal Toxicity With Continuous Nevirapine in Pregnancy. 2004;36(3):1–5. Available from: papers2://publication/uuid/D690B9B1-7395-4171-866C-C5CCAC8F7B99

28. Kovari H, Sabin CA, Ledergerber B, Ryom L, Reiss P, Law M, et al. Antiretroviral Drugs and Risk of Chronic Alanine Aminotransferase Elevation in Human Immunodeficiency Virus (HIV)-Monoinfected Persons: The Data Collection on Adverse Events of Anti-HIV Drugs Study. Open forum Infect Dis. 2016;3(1):ofw009.

29. Martínez E, Blanco JL, Arnaiz J a, Pérez-Cuevas JB, Mocroft a, Cruceta a, et al. Hepatotoxicity in HIV-1-infected patients receiving nevirapine-containing antiretroviral therapy. AIDS. 2001;15(10):1261–8.

30. Rivero A, Mira JA, Pineda JA. Liver toxicity induced by non-nucleoside reverse transcriptase inhibitors. J Antimicrob Chemother. 2007;59(3):342–6.

31. Mira J a, Macías J, Girón-González J a, Merino D, González-Serrano M, Jiménez-Mejías ME, et al. Incidence of and risk factors for severe hepatotoxicity of nelfinavir-containing regimens among HIV-infected patients with chronic hepatitis C. J Antimicrob Chemother [Internet]. 2006;58(1):140–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16720565

32. Bruno R, Sacchi P, Maiocchi L, Zocchetti C, Filice G. Hepatotoxicity and nelfinavir: A meta-analysis. Clin Gastroenterol Hepatol. 2005;3(5):482–8.

33. Peters PJ, Stringer J, McConnell MS, Kiarie J, Ratanasuwan W, Intalapaporn P, et al. Nevirapine-associated hepatotoxicity was not predicted by CD4 count ≥250 cells/μL among women in Zambia, Thailand and Kenya. HIV Med. 2010;11(10):650–60.

6.0 APPENDECES

6.1 Appendix 1: Wits Ethics Clearance Letter

R14/49 Mrs Elizabeth Kamwendo Kachingwe

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M151168

NAME: (Principal Investigator)	Mrs Elizabeth Kamwendo Kachingwe
DEPARTMENT:	Public Health Lilongwe, Malawi
PROJECT TITLE:	Pattern of Transaminase Abnormality in Human Immune Deficiency Virus and Hepatitis B Virus Co-Infected Women on ART in Lilongwe Malawi
DATE CONSIDERED:	27/11/2015
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Charles Chasela
APPROVED BY:	Professor P Cleaton-Jones,Chairperson, HREC (Medical)
DATE OF APPROVAL:	18/01/2016

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004, 10th floor, Senate House/2nd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

6.2 Appendix 2: Malawi Ethics Approval

Telephone: + 265 789 400
Facsimile: + 265 789 431
e-mail doccentre@malawi.net
All Communications should be addressed to:
The Secretary for Health and Population

In reply please quote No. MED/4/36

MINISTRY OF HEALTH
P.O. BOX 30377
LILONGWE 3
MALAWI

21 May 2007

CHARLES CHASELA
UNIVERSITY COLLEGE OF DUBLIN
IRELAND

Dear Madam,

RE: PROTOCOL # 437: EPIDEMILOGY OF HEPATITIS B, C VIRUS CO INFECTION WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION AMONG PREGNANT WOMEN IN LILONGWE,MALAWI.

Thank you for the above titled proposal that you submitted to the National Health Sciences Research Committee (NHSRC) for review. Please be advised that the NHSRC has **reviewed** and approved the study.

APPROVAL NUMBER : 437

The above details should be used on all correspondences, consent forms and documents as appropriate.

- **APPROVAL DATE : 21ST MAY 2007**
- **EXPIRATION DATE 2Oth MAY 2008**

  After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the NHSRC Secretariat should be submitted one month before the expiration date for continuing review.
- **SERIOUS ADVERSE EVENT REPORTING:** All serious problems having to do with subject safety must be reported to the NHSRC within 10 working days using standard forms obtainable from the NHSRC Secretariat.
- **MODIFICATIONS**: Prior NHSRC approval using forms obtainable from the NHSRC Secretariat is required before implementing any changes in the protocol (including changes in the consent documents). You may not use any other consent documents besides those approved by the NHSRC.
- **TERMINATION OF STUDY**: On termination of a study, a report has to be submitted to the NHSRC using standard forms obtainable from the NHSRC Secretariat.
- **QUESTIONS**: Please contact the NHSRC on telephone number *+265 1 789 400/314* or by email on doccentre@malawi.net.
- **OTHER**: Please be reminded to send in copies of your final research results for our records (Health Research Database).

Kind regards from the NHSRC Secretariat.

.....

For: **CHAIRPERSON, NATIONAL HEALTH SCIENCES RESEARCH COMMITTEE**
Promoting Ethical Conduct of Research

Executive Committee: *Dr C. Mwansambo (Chairperson), Prof. E. Molyneux (Vice-Chairperson)*
Registered with the USA Office for Human Research Protections (OHRP) as an International IRB
IRB Number IRB00003905 FWA00005976

6.3 Appendix 3: HREC-90-06-Chasela Approval Letter

11th June 2007

Mr Charles Chasela

c/o Professor Patrick Wall

UCD School of Public Health & Population Science

Woodview House

Belfield

Dublin 4

RE: HREC-90-06-Chasela – Epidemiology of Hepatitis B and C virus and human immunodeficiency virus

Dear Mr Chasela

Thank you for your response to the Human Research Ethics Committee (24/06/07). The decision of the Committee is as follows:

- **As you have met the Committee's requirement, namely that you have obtained local ethical approval, the Committee has approved this study. However, we require a copy of the protocol that was approved and approval is based on your strict adherence to the protocol as approved by the Ministry of Health in Malawi. Any amendments, additions or revisions must be approved locally (Ministry of Health, Malawi) and notified to the UCD Human Research Ethics Committee.**

Approval is time limited – any extensions to the original timeframe for the

approved study will need to be approved by the Committee. Any changes to the original proposal will need to be approved by the Committee. The Committee should be notified of any adverse events that occur during the conduct of your research.

If you have any queries please contact the Research Ethics Office.

Yours sincerely,

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Dr Michelle Butler

Chairman, Human Research Ethics Committee